From Point A to Point B, and What it Means for Epilepsy

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A New Projection From the Deep Cerebellar Nuclei to the Hippocampus *via* the Ventrolateral and Laterodorsal Thalamus in Mice

Bohne P, Schwarz MK, Herlitze S, Mark MD. *Front Neural Circuits*. 2019;13:51. doi:10.3389/fncir.2019.00051. eCollection 2019.

The cerebellar involvement in cognitive functions such as attention, language, working memory, emotion, goal-directed behavior, and spatial navigation is constantly growing. However, an exact connectivity map between the hippocampus and cerebellum in mice is still unknown. Here, we conducted a tracing study to identify the sequence of transsynaptic, cerebellar–hippocampal connections in the mouse brain using combinations of recombinant adeno-associated virus (rAAV) and pseudotyped deletion-mutant rabies (RABV) viruses. Stereotaxic injection of a primarily anterograde rAAV-WGA (wheat germ agglutinin)-Cre tracer virus in the deep cerebellar nuclei (DCN) of a Cre-dependent tdTomato reporter mouse resulted in strong tdTomato labeling in hippocampal CA1 neurons, retrosplenial cortex (RSC), rhinal cortex (RC), and thalamic and cerebellar areas, whereas hippocampal injections with the retrograde tracer virus rAAV-TTC (tetanus toxin C fragment)-eGFP displayed eGFP positive cells in the RC and subiculum (S). To determine the sequence of mono-transsynaptic connections between the cerebellum and hippocampus, we used the retrograde tracer RABVΔG-eGFP(EnvA). The tracing revealed a direct connection from the dentate gyrus (DG) in the hippocampus to the RSC, RC, and S, which are monosynaptically connected to thalamic laterodorsal and ventrolateral areas. These thalamic nuclei are directly connected to cerebellar fastigial, interposed (IntP), and lateral nuclei, discovering a new projection route from the fastigial to the laterodorsal thalamic nucleus in the mouse brain. Collectively, our findings suggest a new cerebellar–hippocampal connection via the laterodorsal and ventrolateral thalamus to RSC, RC, and S. These results strengthen the notion of the cerebellum's involvement in cognitive functions such as spatial navigation via a polysynaptic circuitry.

Anatomical and Physiological Foundations of Cerebello-Hippocampal Interaction

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Multiple lines of evidence suggest that functionally intact cerebello–hippocampal interactions are required for appropriate spatial processing. However, how the cerebellum anatomically and physiologically engages with the hippocampus to sustain such communication remains unknown. Using rabies virus as a retrograde transneuronal tracer in mice, we reveal that the dorsal hippocampus receives input from topographically restricted and disparate regions of the cerebellum. By simultaneously recording local field potential from both the dorsal hippocampus and anatomically connected cerebellar regions, we additionally suggest that the 2 structures interact, in a behaviorally dynamic manner, through subregion-specific synchronization of neuronal oscillations in the 6 to 12 Hz frequency range. Together, these results reveal a novel neural network macroarchitecture through which we can understand how a brain region classically associated with motor control, the cerebellum, may influence hippocampal neuronal activity and related functions, such as spatial navigation.

Commentary

The cerebellum, the beautiful little brain, has long been recognized as a motor structure. However, accumulating evidence shows a role for the cerebellum in cognitive processes, including hippocampal-dependent spatial navigation,[1] and epilepsy,[2] including temporal lobe epilepsy.[3] For example, on-demand optogenetic modulation of Purkinje cells in the cerebellar cortex can stop hippocampal seizures in a rodent model of temporal lobe epilepsy.[4] A major, unresolved, question is *how*. How does the cerebellum influence hippocampal function and stop seizures? Early experiments, examining degenerating fibers or time delays with electrical stimulation suggested that there might be a direct, monosynaptic, connection from the cerebellum to the hippocampus.[3] However, more modern

techniques have failed to find strong evidence for a direct connection between these structures.

To examine possible cerebellar connections to the hippocampal formation, Watson et al injected rabies virus and fluorescent cholera toxin β-subunit (CTb) into the dorsal DG. At a time point after injection that allows for visualization of likely monosynaptic inputs (30 hours postinjection) with rabies, Ctb and rabies labeling was seen in areas with known projections to the hippocampus, including the entorhinal cortex, the supramammillary nucleus of the hypothalamus, and the medial septum/diagonal band of Broca, among others. However, at this time point, no rabies or CTb labeling was seen in the cerebellum, suggesting no direct route from the cerebellum to the hippocampus. Similarly, Bohne et al injected a modified form of the rabies virus, which allows monosynaptic tracing, into the dorsal hippocampus and did not find labeling of cells in the cerebellum, again suggesting no monosynaptic connection from the cerebellum to the hippocampus.

While a lack of a direct connection from the cerebellum to the hippocampus makes it considerably more difficult to discern the pathway(s) by which the cerebellum influences the hippocampus, it is not without potential benefits. Specifically, while studies have shown that modulating the cerebellum can inhibit seizures, the pathway is not known—determining intermediary brain regions and cell types supporting the connection may provide important additional targets for therapeutic intervention. We return then to the question of *how*. How does the cerebellum influence the hippocampus?

Given that Watson et al used a version of rabies that is not limited to one synapse, but will continue to travel back through connections, they were able to examine second- and third-order connections to the hippocampus. While no labeling in the cerebellum was seen at the 30-hour postinjection time point, by 48 hours some labeling was already noted in the DCN. This suggests that the shortest route from the cerebellum to the hippocampus may require only one intermediary relay station. Additional labeling in cerebellar nuclei, and cerebellar cortex, was seen with longer postinfection time points. Importantly, their data provide some insight into which portions of the cerebellum are connected to the dorsal DG. Despite cerebellar ascending projections being strongly contralaterally biased, labeling of cerebellar nuclei was seen bilaterally. Of interest, previous work targeting the cerebellar cortex for hippocampal seizures also did not seen any strong laterality in effect.[4] This suggests that even though cerebellar projections overall are generally strongly lateralized, this pattern does not hold for its (indirect) connections with the hippocampal formation. Relatively strong labeling was seen in the central portion of the dentate (aka lateral) cerebellar nuclei, and the caudal portion of the fastigial (aka medial) nuclei, as well as in vestibular nuclei. However, relatively few cells were labeled in the IntP nucleus (which has an intermediate medial–lateral location, situated between the dentate and fastigial nuclei). This suggests that, at least for connections to the specific portion of the hippocampal formation labeled in their study, there is selectivity with regard to which aspects of the cerebellum communicate with the hippocampus.

The segregation of circuitry becomes even more striking when examining rabies labeling within the cerebellar cortex, where strong labeling of Purkinje cells was seen in spatially isolated clusters within the central and paraflocculus portions of the cerebellum, and in particular, in zebrinII-positive bands. Again, this suggests that there is *specificity* in the cerebellar to hippocampus connections. This is important from both basic science and translational perspectives. For example, selective targeting of these specific pathways may allow seizure suppression with reduced side effects. Their data also suggest there may be multiple, segregated, lines of communication which converge on the hippocampal formation. Determining the relative impact of each of these input lines will be an important next step.

While the work by Watson et al provides important insight into cerebellar–hippocampal circuits, their studies were not ultimately designed to answer the question of *how* (if not monosynaptic). Their work illustrates that there is definitely a multisynaptic connection from *specific* areas of the cerebellum to the hippocampus, but it does not determine which of theoretically possible pathways ultimately provide the cerebellum to hippocampus functional connectivity. Answering that question will require dedicated, carefully controlled experiments.

Bohne et al attempted to provide greater examination of cerebellar–hippocampal connectivity circuitry through a series of experiments. Unfortunately, however, the data ultimately fall short, due to difficulties in interpretability. In a first set of experiments, Bohne et al injected AAV8-WGA-Cre bilaterally into the cerebellar nuclei of mice expressing tdTomato in a Cre-dependent manner and harvested tissue months later. Limiting interpretability, WGA-Cre can travel (transynaptically) in both anterograde and retrograde directions,[5,6] the very long wait time (5 months) makes the number of synapses traveled extra difficult to discern,[7,8] AAV8 itself can display strong retrograde expression, and potential leakage into the fourth ventricle was not considered. While interpreting findings is therefore extremely difficult, it is nonetheless notable that labeling was found in CA1 pyramidal neurons (apparently without strong labeling in the DG). This suggests that an area receiving input from or projecting to the cerebellar nuclei may project to or receive input from the CA1 (or an intermediary brain region). The authors also did the reverse study, injecting AAV8-WGA-Cre into the right DG. While many of the same experimental variables again limit interpretability, it is of interest that bilateral labeling of the simplex and lobules 4/5 in the cerebellar cortex was noted. This is of interest as the labeling was bilateral and because these are the same areas recently targeted by optogenetic manipulation to stop hippocampal seizures.[4] Bohne et al also injected AAV8-WGA-Cre into the left cerebellar cortex (targeting CrusI/CrusII) and AAV8-TTC-eGFP (as a retrograde tracer[9]) into the left DG of tdTomato mice, in an attempt to identify potential areas of overlap. However, limited expression of eGFP indicated incomplete labeling

of projections to the hippocampus, no neurons expressed both fluorescent proteins, and, strangely, putative astrocytes were Tomato positive in areas such as the perirhinal cortex, again limiting interpretability of results.

However, as discussed above, in separate experiments, Bohne et al additionally used modified rabies virus to examine monosynaptic inputs to the hippocampus and, importantly, found no evidence for direct connections from the cerebellum. The authors also injected modified rabies into the S, the RSC, or the RC and found labeling of putative inputs in the dorsomedial laterodorsal, the ventrolateral laterodorsal, and the ventrolateral thalamic nuclei in all three cases (but, notably, not in the cerebellum). The authors therefore injected modified rabies virus into these thalamic nuclei, and found labeling in the contralateral fastigial, IntP, and dentate cerebellar nuclei. While this suggests that certain areas that project to the hippocampus may receive input from thalamic nuclei that receive input from the cerebellar nuclei, it (1) does not show that the cells in those regions that receive input in turn project to the next step (ie, connections were shown in separate experiments at the general brain region level, in contrast to, eg, the study by Watson et al, which used successive transynaptic labeling), (2) does not address other potential pathways, including potential disynaptic pathways suggested in the work by Watson et al, (3) does not confirm the functionality of putative pathways, and (4) used AAV8 helper viruses (see concerns above) and lacks important controls, including controls for recently described "invisible" TVA expression (ie, TVA expression without observable red fluorescent protein expression).[10] Therefore, while Bohne et al provide an interesting data set, their work ultimately falls short of answering the question of how the cerebellum influences hippocampal activity.

Important questions do not always have easy answers. Work by Watson et al and Bohne et al demonstrate that a monosynaptic connection is unlikely. Determining how the cerebellum influences the hippocampus therefore becomes immediately a more difficult question. Well controlled, carefully executed, studies designed to specifically answer this question will be required to determine what pathway(s) ultimately underlie the functional connectivity observed between the cerebellum and the hippocampus. However, these difficulties do not undermine the importance of the question. Determining how the cerebellum influences the hippocampus is important at a basic science level. It is also important for epilepsy, and may open the door to novel therapeutic strategies. It becomes just a matter of how to get there from here.

By Esther Krook-Magnuson

ORCID iD

Esther Krook-Magnuson https://orcid.org/0000-0002-6119-0165

References

1. Rochefort C, Arabo A, André M, Poucet B, Save E, Rondi-Reig L. Cerebellum shapes hippocampal spatial code. *Science*. 2011; 334(5):385-389.
2. Kros L, Eelkman Rooda OHJ, De Zeeuw CI, Hoebeek FE. Controlling cerebellar output to treat refractory Epilepsy. *Tren neurosci*. 2015;38(12):787-799.
3. Yu W, Krook-Magnuson E. Cognitive collaborations: bidirectional functional connectivity between the cerebellum and the hippocampus. *Fron Syst Neurosci*. 2015;9:177.
4. Krook-Magnuson E, Szabo GG, Armstrong C, Oijala M, Soltesz I. Directed optogenetic intervention inhibits spontaneous hippocampal seizures in a mouse model of temporal lobe epilepsy. *eNeuro*. 2014;1(1):pii: e.2014.
5. Gradinaru V, Zhang F, Ramakrishnan C, et al. Molecular and cellular approaches for diversifying and extending optogenetics. *Cell*. 2010;141(1):154-165.
6. Fabian RH, Coulter JD. Transneuronal transport of lectins. *Brain Res*. 1985;344(1):41-48.
7. Libbrecht S, Van den Haute C, Malinouskaya L, Gijsbers R, Baekelandt V. Evaluation of WGA-Cre-dependent topological transgene expression in the rodent brain. *Brain Struct Funct*. 2017;222(2):717-733.
8. Martinelli DC, Chew KS, Rohlmann A. Sudhof, expression of C1ql3 in discrete neuronal populations controls efferent synapse numbers and diverse behaviors. *Neuron*. 2016;91(5):1034-1051.
9. Maskos U, Kissa K, St Cloment C, Brûlet P. Retrograde transsynaptic transfer of green fluorescent protein allows the genetic mapping of neuronal circuits in transgenic mice. *Proc Natl Acad Sci U S A*. 2002;99(15):10120-10125.
10. Hafner G, Witte M, Guy J, et al. Staiger, mapping brain-wide afferent inputs of parvalbumin-expressing GABAergic neurons in barrel cortex reveals local and long-range circuit motifs. *Cell Rep*. 2019;28(13):3450-3461 e3458.